



## Evaluation of Topramezone and Benzobicyclon for Activity on Giant Salvinia

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**PURPOSE:** The purpose of this research was to evaluate the recently registered aquatic herbicide, topramezone, and the experimental herbicide, benzobicyclon, for activity against the invasive floating fern giant salvinia (*Salvinia molesta* Mitchell).

**BACKGROUND:** Giant salvinia is a free-floating, mat-forming aquatic fern native to southeastern Brazil (Forno and Harley 1979) that has become problematic in water bodies throughout the southeastern U.S., Puerto Rico, and Hawaii (Mudge et al. 2013). Under optimal growth conditions, plants can double in coverage every 36 to 53 hr (Cary and Weerts 1983; Johnson et al. 2010). The surface of the floating fronds are covered by rows of white, bristly hairs (trichomes), topped with four branches united distally to form a structure resembling an “eggbeater” (McFarland et al. 2004), which can impede herbicide deposition and penetration (Nelson et al. 2007). Giant salvinia initially expands throughout an aquatic system in the primary growth or colonizing stage; it progresses through the secondary growth stage; and it finally reaches maximum capacity in a single mat-forming layer, otherwise known as the tertiary growth stage (Oliver 1993). Dense infestations disrupt transportation, hinder water uses, impact desirable native plant communities, and increase mosquito breeding habitat (Jacono 1999; Jacono and Pitman 2001; Nelson et. al 2001). Since 1999, giant salvinia has become especially problematic in reservoirs, lakes, ponds, rivers, and bayous throughout Texas and Louisiana (Owens et al. 2004, Johnson et al. 2010).

Natural resource managers have attempted chemical, biological, mechanical, and physical control methods to manage giant salvinia (Madsen and Wersal 2009), with chemical and biological control being more widely used in the U.S. The Louisiana Department of Wildlife and Fisheries (LDWF) spent over \$3,000,000 to manage over 14,979 ha of giant salvinia by chemical control measures (herbicides, labor, and application costs) in 2013 (A. Perret, personal communication 2014). In 2014 and 2015, the U.S. Army Corps of Engineers (USACE) Fort Worth District spent approximately \$300,000 and \$400,000 on aquatic herbicides and the release of the giant salvinia weevil (*Cyrtobagous salviniae*) to manage approximately 121 and 1,012 ha, respectively, on two USACE reservoirs in Texas (A. Gray, personal communication 2015). On an annual basis, the majority of giant salvinia infestations managed in Louisiana and Texas are treated with a combination of the aquatic herbicides glyphosate and diquat, with the inclusion of two adjuvants (nonionic surfactant with buffering agents and a nonionic organosilicone surfactant) in a single tank mix (Mudge et al. 2014; Mudge et al. 2016).

Due to concerns stemming from the development of herbicide resistance, herbicides with different modes of action should be rotated, if available, as a resistance-management practice. Therefore,

evaluation of other chemistries for giant salvinia management is needed. Topramezone {[3-(4,5-dihydro-isoxazol-3-yl)-4-methylsulfonyl-2-methylphenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone} is labeled for post-emergence weed control in field corn, sweet corn, and popcorn (*Zea mays* L.) ((PMRA (Pest Management Regulatory Agency)) 2006). The 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, topramezone, is a bleaching herbicide that targets a plant-specific enzyme. Topramezone also possesses a low toxicity to mammals, fish, and invertebrates (WSSA 2007), making it a good candidate for aquatic uses. In 2013, topramezone was registered for aquatic sites by the U.S. Environmental Protection Agency (USEPA) to control submersed, floating, and emergent invasive vegetation (University of Florida 2015). Limited research has been published on the efficacy of topramezone. Glomski and Netherland (2011) utilized a small-scale primary screening method to evaluate topramezone efficacy and selectivity against Eurasian watermilfoil (*Myriophyllum spicatum* L.), hydrilla (*Hydrilla verticillata* (L.f.) Royle), coontail (*Ceratophyllum demersum* L.), elodea (*Elodea canadensis* Michx.), Southern naiad (*Najas quadrangularis* (Spreng.) Morong.), wild celery (*Vallisneria americana* Michaux), and other species. Johnson et al. (2010) screened topramezone for activity against giant salvinia; however, this herbicide was not identified as providing >90% control and the rates, plant growth stage, or timing were not identified in the research article. Benzobicyclon {3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylsulfanyl)bicyclo[3.2.1]oct-3-en-2-one}, also a HPPD inhibitor, was registered in Japan (2001) as a rice herbicide and is currently being developed for rice weed control in the U.S. (Heiser 2014; Komatsubara et al. 2009). Benzobicyclon is efficacious against common rice weeds, including barnyardgrass (*Echinochloa crus-galli* L), yellow nutsedge (*Cyperus esculentus* L.), and ducksalad (*Heteranthera limosa* (Sw.) Willd.) (McKnight et al. 2016). Currently, no research has been published on the activity of benzobicyclon on aquatic weeds, and whether the registrant has the intent to pursue a Section 3 aquatic registration in the U.S. is unknown.

Aquatic weeds pose threats to many ponds, lakes, rivers, reservoirs, and bayous. Some of these water weeds, including giant salvinia, can also be found in agricultural settings such as rice fields, crawfish ponds, and the irrigation canals used to supply water to these fields. Registration of herbicides in various markets allow for expanded use to combat giant salvinia and other weeds that crossover between aquatic and agricultural settings. Due to the limited number of herbicides registered in the U.S. for aquatic weed control, the need to develop resistance management strategies, and the acceptable toxicological profiles exhibited by topramezone and benzobicyclon, these two herbicides were selected for efficacy screening against giant salvinia.

**MATERIALS AND METHODS:** An outdoor mesocosm trial was conducted at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility, in Baton Rouge, LA, to evaluate the efficacy of topramezone and benzobicyclon against mature giant salvinia populations. Trials were initiated in September 2014 and May 2015 (hereafter referred to as fall and spring trials, respectively). Giant salvinia used in this research was collected from cultures maintained at the LSU Aquaculture Research Station. Equal amounts of fresh plant material, enough to cover approximately 75% of the water surface, were placed inside 76 L plastic containers (49.5 cm diameter by 58.4 cm height). The containers were filled with pond water that was amended with Osmocote® fertilizer (The Scotts Company, Marysville, Ohio, 19-6-12, 116.7 mg L<sup>-1</sup>) initially and 4 weeks after herbicide application to provide adequate nutritional growth conditions for the plant populations. Water volume in the containers was maintained weekly at 60 L. The containers were placed inside larger plastic tanks (1136 L) partially filled with water to act as a water bath to regulate water temperature

in the treatment containers. Culture techniques were adapted from previous giant salvinia research (Nelson et al. 2001; Nelson et al. 2007; Mudge et al. 2012).

Plants were allowed to grow and acclimate to container conditions for 2 weeks prior to herbicide application. At the time of herbicide treatment, the mature plants had reached 100% coverage in each container and were 1 to 2 plant layers thick, with a mean dry weight of  $20.5 \pm 1.5$  and  $20.8 \pm 0.3$  g for the fall and spring trials, respectively. A non-treated control was included. Treatments were randomly assigned and replicated four times. Topramezone (Oasis®, SePRO Corporation, Carmel, Indiana) was applied at the maximum foliar ( $392.5$  g active ingredient (a.i.)  $\text{ha}^{-1}$ ) and subsurface application rates ( $0.05$  mg a.i.  $\text{L}^{-1}$ ). Benzobicyclon (Gowan Company, LLC, Yuma, Arizona) was applied at foliar and subsurface rates of  $560.7$  g a.i. and  $1$  mg a.i.  $\text{L}^{-1}$ , respectively. Foliar treatments were applied using a forced air  $\text{CO}_2$ -powered sprayer at an equivalent of  $935 \text{ L ha}^{-1}$  diluent delivered through a single TeeJet® (Spraying Systems Co., Wheaton, Illinois) 80-0067 nozzle at  $20$  psi. Subsurface treatments were pipetted from a concentrated stock solution into the water column of each container. Visual estimates of giant salvinia injury were recorded every day for the first 2 weeks and weekly thereafter to determine speed and long-term effectiveness of herbicide treatments.

At 8 weeks after treatment, (WAT) (November 2014 and July 2015), all viable giant salvinia biomass was harvested, dried to a constant weight ( $60^\circ\text{C}$  for 1 week), and recorded as dry weight biomass. A two-way analysis of variance (ANOVA) determined a treatment by trial interaction; therefore, fall and spring data were not pooled. Data were subjected to ANOVA and means separated using Student-Newman-Keuls (SNK) method ( $p \leq 0.05$ ).

**RESULTS AND DISCUSSION:** Topramezone resulted in faster injury symptoms to giant salvinia compared to benzobicyclon regardless of application method. Since both herbicides are HPPD inhibitors, the primary injury symptom observed was chlorosis, which was initially noted in the younger/newer fronds by 3 WAT. Injury was initially observed on plants treated with a foliar application of topramezone 1 WAT, whereas topramezone subsurface and benzobicyclon foliar and subsurface required 2 to 3 weeks before injury was observed. When applied to the submersed plant hydrilla, topramezone is absorbed into the plant tissue and symptoms generally first appear in 7-10 days (University of Florida 2015), which is similar to the findings in the current research. Both herbicides produced faster and more intense bleaching symptoms in the spring application period compared to the fall. The slow development of injury was likely due to decreased plant growth rate in the fall. Eventually, injury was noted in the older fronds 3 to 4 WAT. With regard to the fall trial, severe injury (necrosis) or plant decomposition failed to occur, and plants continued to exhibit chlorosis with minimal recovery until harvest at 8 WAT. The lack of severe injury is likely attributed to time of year when plant growth is typically less during the fall compared to the spring.

Following the fall herbicide application, all herbicide treatments reduced giant salvinia dry weight 28 to 42% of the non-treated control by 8 WAT (Figure 1). There were no differences between foliar or subsurface topramezone and benzobicyclon treatments for the fall treatment. Conversely, all treatments, except topramezone subsurface, were efficacious following a spring treatment; reducing plant dry weight 55 to 99% when compared with the untreated control. Giant salvinia treated with foliar and subsurface topramezone and benzobicyclon applications in the fall were less responsive compared to plants treated in the spring. Previous giant salvinia research conducted at various application timings (spring, summer, and fall), indicated plants treated in the fall were slower to

respond (i.e., injury symptoms) to glyphosate, diquat, flumioxazin, carfentrazone, and endothall (Mudge et al. 2016). These same herbicides were also less efficacious when applied in the fall compared to the spring or summer application timings (Mudge et al. 2016). The lack of giant salvinia control by the subsurface topramezone treatment was not unexpected. When managing hydrilla, topramezone is typically applied at 30 to 50  $\mu\text{g}$  a.i.  $\text{L}^{-1}$  and maintained at or near the initial concentration for a minimum of 60 days (University of Florida 2015). Giant salvinia was initially treated at the maximum rate, but a secondary or follow-up treatment was not administered to maintain concentrations near this level, which likely allowed plants to recover.

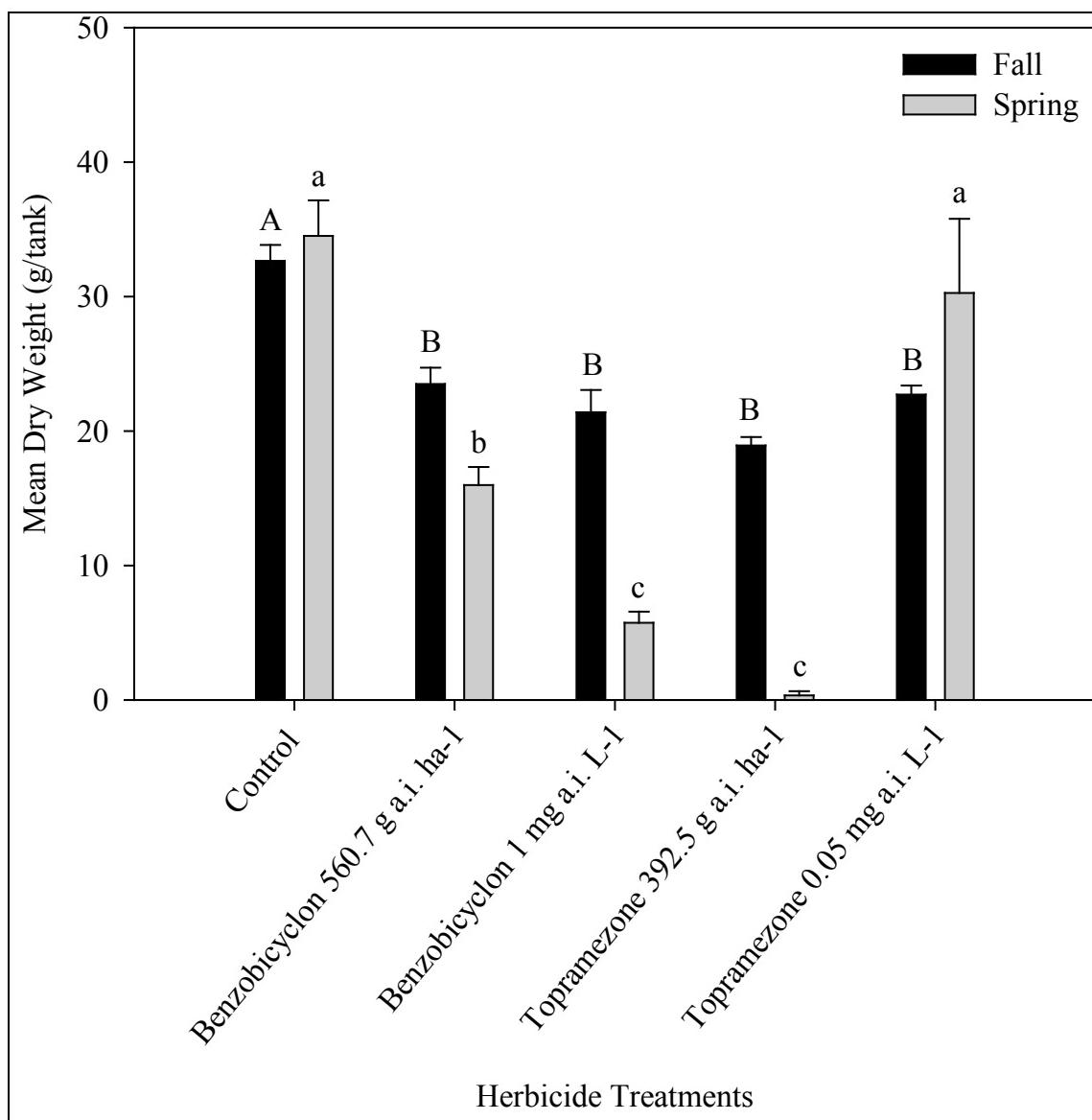


Figure 1. Effect of foliar ( $\text{g a.i. ha}^{-1}$ ) and subsurface ( $\text{mg a.i. L}^{-1}$ ) benzobicyclon and topramezone applications on giant salvinia dry weight (g) in the fall 2014 and spring 2015, eight weeks after treatment. Pre-treatment biomass was 20.5 and 20.8 for the fall and spring trials, respectively. Treatments with the same letter are not significant according to Student-Newman-Keuls method at  $p \leq 0.05$ ;  $n = 4$ .

The spring-applied foliar topramezone treatment at 392.5 g a.i. ha<sup>-1</sup> and benzobicyclon subsurface at 1 mg a.i. L<sup>-1</sup> were the most efficacious treatments and were statistically similar. These results provide evidence that foliar applications of topramezone may be suitable for managing giant salvinia, and can provide users with an alternate mode of action to mitigate potential herbicide resistance. In addition, these data demonstrated that benzobicyclon, as a subsurface application, is efficacious against a difficult-to-control floating plant and warrants additional screening as an aquatic herbicide. Results were expected given the fact that benzobicyclon is applied in water (flooded conditions) in rice cropping systems to achieve effective weed control (McKnight et al. 2016). In addition, McKnight et al. (2015) found that control and activity increases in deeper water and that the flood must be maintained to optimize herbicide efficacy.

**FUTURE WORK:** These data provided evidence that topramezone, when applied as a foliar treatment, is highly efficacious against giant salvinia. Field trials should be conducted to evaluate giant salvinia control on an operational scale to verify and validate observations from small-scale studies. In addition, benzobicyclon should be screened for activity on other floating, emergent, and submersed invasive aquatic plants.

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